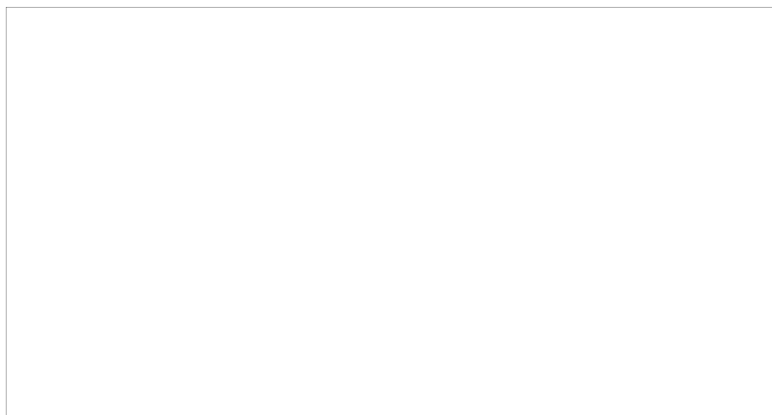


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ON THE PARTICIPATION OF ALDURONIC ACIDS  
IN THE PROCESS OF PHOTOSYNTHESIS

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 the USSR. Presented by Academician  
 A. I. Oparin on May 4, 1950).

[A Digest]

We have shown in previous work (1) that alduronic acids, which are unstable and react with hydroxylamine forming hydroxamic acids, are present in green leaves. We have established that the compounds in question are actually alduronic acids by isolating the hydroxamic acids, treating them with alkali so that ammonia was split off, and identifying the remaining carbohydrate residue according to C. Neuberg (3). This result induced us to investigate in greater detail the part which alduronic acids play in photosynthesis.

We determined the content of alduronic acids in the fraction which, according to S. Ruben et al, (2), contains the carbon isotope used as a tracer after fixation in the light of carbon dioxide containing the tracer. 10 g. of leaves were placed into 100 ml. of boiling water. After 2 min. of boiling the leaves were triturated in the water and their juice was pressed out. 4 g. of talcum were stirred up with the juice, whereupon the mixture was filtered under suction. The clear filtrate was extracted three times

with ether (25 ml. portions) and after that 3 times with isocetyl alcohol (20 ml. portions). The aqueous solution was evaporated in vacuum down to a volume of 5 ml., and the small quantity of precipitate formed during evaporation was removed by centrifuging. When 25 ml. of 96% ethyl alcohol were added to the aqueous solution, a precipitate which was insoluble in the alcohol (now having a concentration of 80%) has formed (fraction A.) Upon removal of fraction A, the filtrate was precipitated with a saturated solution of barium chloride in 80% alcohol. The precipitate formed upon addition of barium chloride (fraction B) was isolated by centrifuging, washed with 96% alcohol, washed with ether, and then dried. Fraction B was tested for the presence of uronic acids by the reaction with naphthoresorcinol (1, 3-naphthalenediol) and subsequent extraction with benzene (3). The leaves of primrose, wheat, white clover, and portweed (*Petasoteton Tourn.*) were tested in this manner. Fraction B revealed the presence of uronic acids in every instance. The reactions for pyruvic acid, with salicylic aldehyde, and for glyoxylic acid according to Fosse (7) were negative.

According to Gaffron et al (4), fractions containing tracer carbon upon fixation of carbon dioxide in the light undergo no changes in the dark while they are easily modified when exposed to illumination. E. A. Boyshenko (5) showed that it is possible to reduce with hydrogen carbon dioxide subsequently to the latter's fixation by the chloroplasts.

We assumed that the alduronic acids are formed as a result of the fixation of carbon dioxide and checked this assumption in the following manner. Two samples of the leaves, weighing 10 g. each, were placed into air containing 10% carbon dioxide and into air not containing any carbon dioxide (or into hydrogen which was constantly renewed for the purpose of eliminating carbon dioxide formed by respiration) respectively. The first sample was kept in complete darkness, while the second was illuminated by a 500 candle

power source placed at a distance of 20 cm. under employment of water cooling. The temperature in both cases was kept at 18-20°C. After expiration of a certain period of time (see tabulated data), the leaves were rapidly transferred into 100 ml. of boiling distilled water. Boiling was continued for 2 minutes and then fraction B was obtained according to the method described above. Fraction B was dissolved in 2 ml. of water which had been acidified with HCl and 0.5 ml. of the solution obtained in this manner were tested for alduronic acids by the naphthoresorcinol reaction followed by extraction with benzene. The data obtained are presented in Table 1.. In individual experiments, the benzene extracts were compared by means of a stage photometer. The table lists extinctions obtained in cuvettes (cells) 0.25 cm. thick with an 8-53 filter interposed.

It can be seen that the alduronic acids which are always present in fraction B are not altered by storing the leaves in the dark, but disappear partially or completely on illumination of the leaves in the absence of carbon dioxide, i.e. under conditions which promote the reduction of the carboxyl group.

Further experiments were carried out with wheat sprouts. Upon sprouting in the dark, the seedlings were divided into two portions. One of these portions was allowed to grow further in the dark, while the other was exposed to daylight. After the seedlings had become completely green in the light, i.e. photosynthesis had begun, both groups of seedlings were fixed by placing them into boiling water. After 2 minutes of boiling the sprouts were removed from the water and the weakly colored aqueous extract was condensed in vacuum, whereupon fraction B was obtained from it. In some experiments the aqueous extracts were precipitated with neutral lead acetate in order to eliminate colored substances. In such cases the precipitate was filtered off, the lead was precipitated with hydrogen sulfide, and upon

removal of the hydrogen sulfide fraction B was obtained from the solution. The results obtained in these experiments are listed in Table 2. In the case of Experiment 5, Table 2, the test in fraction B for alduronic acids with thioglycolic acid (6) was positive for the group grown in the light and negative for the group grown in the dark.

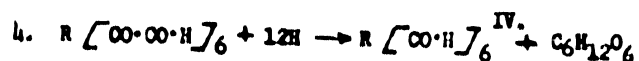
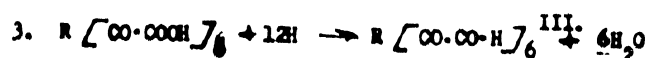
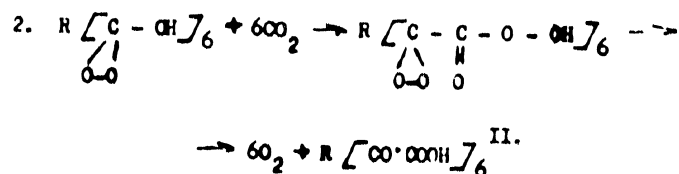
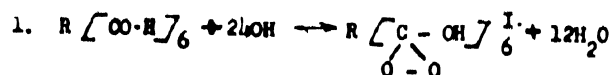
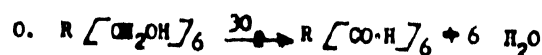
As can be seen from the data of Table 2, in the case of young seedlings which had not been exposed to photosynthesis fraction B was almost totally devoid of alduronic acids. Alduronic acids appear only after the seedlings have become green, i.e. after photosynthesis has started. In other words, the alduronic acids of fraction B form as a result of photosynthesis.

Taking into consideration E. A. Boychenko's work on the possibility of carrying out the initial reactions of photosynthesis with chloroplasts removed from the plants (5), we investigated the presence of alduronic acids in the fraction B obtained from isolated chloroplasts. Undamaged chloroplasts were obtained according to Boychenko in the form of films on filter paper. Before the experiment the sheets of paper were extracted three times with water at 100°C. The chloroplast films were placed into boiling water for 2 minutes. Fraction B was obtained from the filtered aqueous extracts obtained in this manner. The investigation of the fractions B yielded the results summarized in Table 3.

It can be concluded from all of the data obtained that alduronic acids occur in the primary products of carbon dioxide assimilation. These acids remain unchanged in the dark and their quantity drops in the fraction containing the primary products after the plant has been exposed to light in the absence of carbon dioxide. Identical results are obtained with isolated chloroplasts. Exposure to light is necessary in order that alduronic acids form in fraction B of wheat seedlings.

Correlating the results obtained in this instance with our former results on the formation in leaves of unstable compounds which have the pro-

perities of peroxides and form hydroxamic acids under the action of hydroxylamine, these hydroxamic acids forming alduronic acids upon alkaline hydrolysis, we conclude that alduronic acids probably participate in the process of photosynthesis. Taking into consideration the process of oxidation of polysaccharides into polyuronic acids and assuming that this oxidation takes place with the intermediate formation of peroxides, one must assume that the unstable bonds of peroxides are a determining factor in the photochemical fixation of carbon dioxide, and bring about this fixation. The participation of alduronic acids in photosynthesis can be represented as follows:



R is a complex carbohydrate residue which may be combined with non-carbohydrate groups. Formation of I explains the reaction of the undamaged leaf with hydroxylamine. Fraction B contains product II, which gives the alduronic acid reaction. The disappearance of uronic acids in fraction B, which takes

place under the conditions of reduction <sup>is carrying</sup> ~~taking place~~ during exposure to light, is explained by reaction 3. The intermediate product III induces the reduction processes occurring during the fixation of carbon dioxide by chloroplasts in an atmosphere of hydrogen, as described by Boychenko.

This hypothesis leads to the idea of a carbohydrate catalyst ["stand" in the original] which would be the compound indicated by IV in the reaction scheme. The presence of any such catalyst would explain why among all the possible stereoisomers of a simple sugar only ~~one~~ products having a definite configuration are formed in photosynthesis. In the process of synthesis, IV is continually regenerated and enters into the reaction again. The "zero" reaction only starts the photosynthesis and requires the presence of oxygen. The difficulty with which reactions 1 and 2 would proceed in the presence of light explains our observations on the reduced amount of peroxides and almost complete absence of alduronic acids in fraction B of seedlings which had not been exposed to light.

Submitted on April 10, 1950

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Table 1.

No. of experiment	Plant tested.	Atmosphere in which plant was exposed to light.	Time of exposure.	Collection of leucans in test for ultraviolet acids contained in fraction 1.	
				Light Experiments in the dark.	Light Experiments in the light.
1	Primrose.	CO <sub>2</sub> free air	1 h.	No coloration	Red-violet.
2	"	" " "	1 h.	No coloration, 0.05	Red-violet, 0.10
3.	White clover	Hydrogen	1 h.	Traces of color, 0.05	Strong red-violet, 0.15
4	" "	"	1 h. 30 m.	Weak red-violet	Strong red-violet
5	Pansy	Boiled water, stream of hydrogen	2 h.	Weak yellow coloration	Strong red-violet

Table 2.

Number of experiment	Days elapsed until sprouting	Type of treatment	Weight of fraction B obtained in mg.		Coloration of fraction B, total for amino acids contained in fraction B.	
			Experiments in the light	Experiments in the dark	Experiments in the light	Experiments in the dark
1	10	Fractionation of the aqueous extract	77.5	26.5	Strong red-violet coloration	Colorless
2	8	After precipitation with lead	12.0	5.6	Ditto	Trace of color
3	8	Ditto	10.8	6.0	Ditto	" " "
4	7	Fractionation of the aqueous extract	24.8	12.0	Red-violet, 0.59	Weak yellow, 0.28
5	10	Ditto	45.6	26.4	Strong red-violet, 0.27	Yellow-brown, 0.35

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Table 3.

Number of experiment	Plant from which chloroplasts have been isolated	Conditions to which the chloroplasts were exposed	Quantity of fraction II obtained	Collection of benzene in test for chlorophyll acids contained in fraction II
1	Primrose, 30 g.	Freshly isolated chloroplasts	Small quantity of precipitate. Was not weighed.	Not visible
2	White clover, 100 g.	" " "	3.6 mg.	Weak red-violet
	Control experiment with filter paper	" " "	Trace	No coloration
3	Tobacco, 55 g.	" " "	Small quantity of precipitate	Weak red-violet
4	White clover, 100 g. Isolated chloroplasts were divided into 2 equal portions. One of the portions was exposed for 1 h. 30 m. to conditions a) /see next column/, while the other was exposed for the same length of time to conditions b):	a) Kept in the dark in an atmosphere containing 10% of CO <sub>2</sub> b) Kept in an atmosphere of hydrogen under an illumination equal to 500 candle power	Precipitate was not weighed	Red-violet  No coloration

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